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Stability of Live Attenuated Venezuelan Equine Encephalitis Vaccine

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Reconstituted Venezuelan equine encephalitis vaccine was found to retain significant titers of plaque-forming virus after storage at 4 or 22 C for 24 hr.

An attenuated Venezuelan equine encephalitis (VEE) vaccine was developed at the U.S. Army Medical Research Institute of Infectious Diseases for protection of at-risk personnel (1). Conditions of administration of the vaccine to humans have been closely controlled, and even when the vaccine is held at 4 C, it has been recommended that reconstituted product be discarded after 2 hr.

Once safety and efficacy of this product for *Equidae* had been established (3), it was used extensively for vaccination of horses in Central America and the United States (R. O. Spertzel and R. W. McKinney, Mil. Med., *in press*).

Whereas discarding vaccine 2 hr after reconstitution may be a reasonable requirement for immunization of humans, during a vaccination campaign in horses, it would be advantageous to use vaccine reconstituted for longer than 2 hr. Further, the temperature at which vaccine is held prior to administration cannot be as rigidly controlled under field conditions as in a laboratory or hospital.

To establish more practical guidelines for use of vaccine in the field, the effect of temperature, protein concentration, and time upon stability of VEE vaccine was investigated.

Each vial of VEE vaccine—live, attenuated, dried (lot 5, run 3)—produced by National Drug Co., Swiftwater, Pa., was reconstituted with 1.2 ml of sterile water for injection, USP. Commercial diluent, a modified Hanks balanced salt solution containing 0.5% human serum albumin (HSA), USP, prepared by the same company, was combined with 25% HSA to additional final concentrations of 1, 2, and 4% (v/v). The reconstituted vaccine was diluted 1:50 with the stated concentration of HSA in diluent, and samples were incubated at 4, 22, and 37 C.

Samples were collected by removing 1-ml amounts at predetermined times and storing them at −60 C. For assay, samples were thawed and serially diluted in phosphate-buffered saline with 1% normal rabbit serum, *p*H 7.3. One milliliter of each 10-fold dilution was inoculated in duplicate on monolayers of primary chick embryo cell culture. After viral adsorption at 35 C for 1 hr, the cells were overlaid with 25 ml of medium 199 with Earle's balanced salt solution containing a final concentration of 1.5% Ion agar. After incubation at 37 C for 72 hr, 5 ml of a 1:7,500 concentration of neutral red in Earle's balanced salt solution was added to each flask. The flasks were incubated for an additional 3 hr, after which plaques were counted.

When the vaccine was reconstituted as recommended to a final dilution of 1:50, the mean titer of virus in 12 randomly chosen vaccine vials was $1.31 \pm 0.09 \times 10^5$ plaque-forming units (PFU)/ml. The per cent of surviving PFU of virus as a function of incubation time, HSA concentration, and temperature is presented in Fig. 1. Lines were fitted to the data by the method of least squares; no statistical differences could be detected among the range of HSA concentrations tested; i.e., 0.5% HSA was as effective a stabilizer as 4.0% HSA.

In light of these findings, the data from all of the HSA concentrations at a single temperature was grouped and a line was fitted to these data (line not shown). The results indicated that no difference could be detected between inactivation rates at 22 and 4 C, although the data points derived at 22 C were more scattered than those derived at 4 C. In both cases, more than $6.5 \cdot 10^4$ PFU/ml remained after 8 to 10 hr. The slope of the line derived from the data for 37 C was significantly steeper than that seen at the other temperatures, but the loss of only 50% of the original infectivity at 22 or 4 C in 8 to 10 hr and the loss of only one order of magnitude of infectivity after 24 hr at the same temperatures demonstrated the extreme thermal sta-

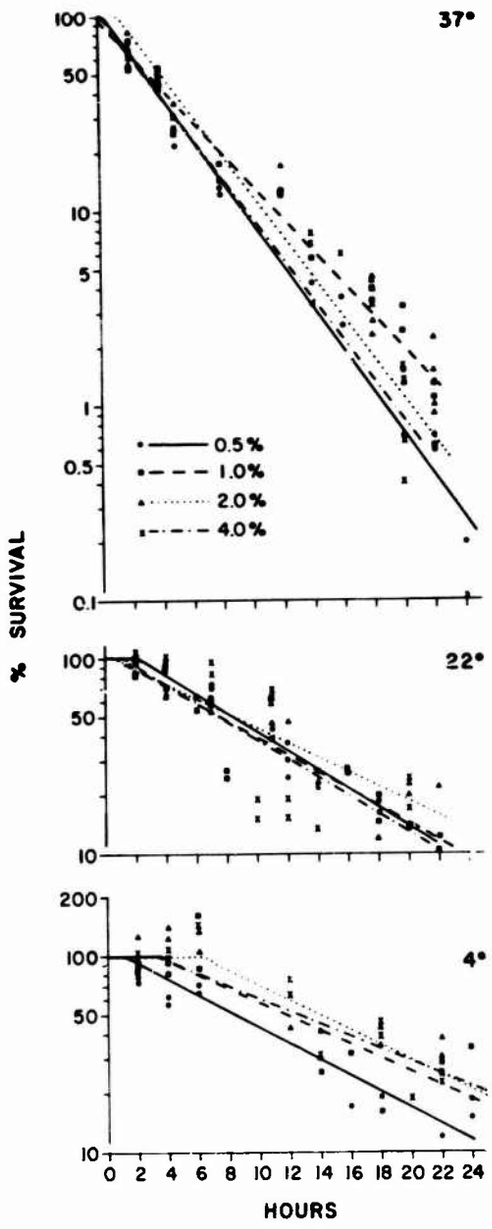

FIG. 1. *Per cent survival of virus as a function of human serum albumin concentration and time at specified temperatures.*

bility of this attenuated strain of VEE virus.

Based on these findings and those of Spertzel and Kahn (3) it is conservatively estimated that the virus titer of reconstituted vaccine held at 4 or 22 C for 24 hr would be sufficient to immunize *Equidae*.

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